



## A Guide to Understanding and Performing all Appropriate Validation Steps When Adopting a New Endotoxin Testing Reagent

### Limitations of Common Endotoxin Testing Solutions

The adaptation of in-house endotoxin testing for a pharmaceutical or medical device manufacturer can be a daunting task. Many small-volume manufacturers find themselves in one of two common situations. Either they are utilising a contract testing organisation (CTO) and paying a great deal of costs, or they have adopted a solution that is set up by the manufacturer with much of the validation services outsourced.

A potential limitation of these methods is cost. Utilising a CTO is appealing to low-volume manufacturers. However, it can be a limitation to expansion as testing costs increase with volume. A system that does not allow versatility in reagent usage can lock the client into manufacturers' costs and price increases.

However, an even bigger drawback to both situations is the lack of testing control and feedback that the user receives for each method. The disadvantage to the convenience of a contract testing service is the increase in time to results as well as greater limitations on the frequency of testing monitoring. In-house testing can provide results in an hour or less if needed. This nearly real-time feedback on the quality of the manufacturing process is invaluable to the trend monitoring of the manufacturing process.

### Dangers of an Improperly Performed Analytical Methods Validation

Real-time feedback can be provided by seemingly attractive solutions. Some solutions utilise outsourced verification data provided by the manufacturer. This provides the convenience of having preparatory tests being done offsite – outsourcing much of the validation. However, a great limitation of this method is that the routine testing takes place in a different location with different analysts, accessories, and accompanying instruments, which may not fully support the verification test requirements stated in USP chapter 85: "To assure the precision or validity of the turbidimetric and chromogenic techniques, preparatory tests are conducted to verify that the criteria for the standard curve are valid and that the sample solution does not interfere with the test. Validation for the test method is required when conditions that are likely to influence the test result change." Although an outsourced standard curve may technically check the requirements, it assumes that changing "conditions that are likely to influence the test result" is not a factor between the different locations, personnel, equipment, and accessories in and with which the validation testing and the routine testing takes place.

Although routine test monitoring includes the PPC and recovery rates at the testing location, the concern about the offsite verification data is not unfounded. Why would the

USP require suitability verification if redundant information is obtained during routine testing? The information obtained during routine testing is not for validation but to monitor daily test conditions. A validation performed in the same conditions as the actual test, including location and analyst, is imperative when setting up in-house testing.

Invalid results in routine testing presume an accurately validated test method to reveal issues in reagent preparation, contamination, and instrument failure. However, repeated failure of routine results can be caused by improperly determined testing parameters found using data obtained away from the in-house testing location. Being caught in this situation can cause undue stress on the clients as they face regular actionable results to investigate, retest, and report. The challenge of changing testing methods does not present itself as a feasible solution because much time and money are invested in the test method. However, this leads to the crucial blunder of passing off systematic test method errors such as random user mistakes or environmental contamination.

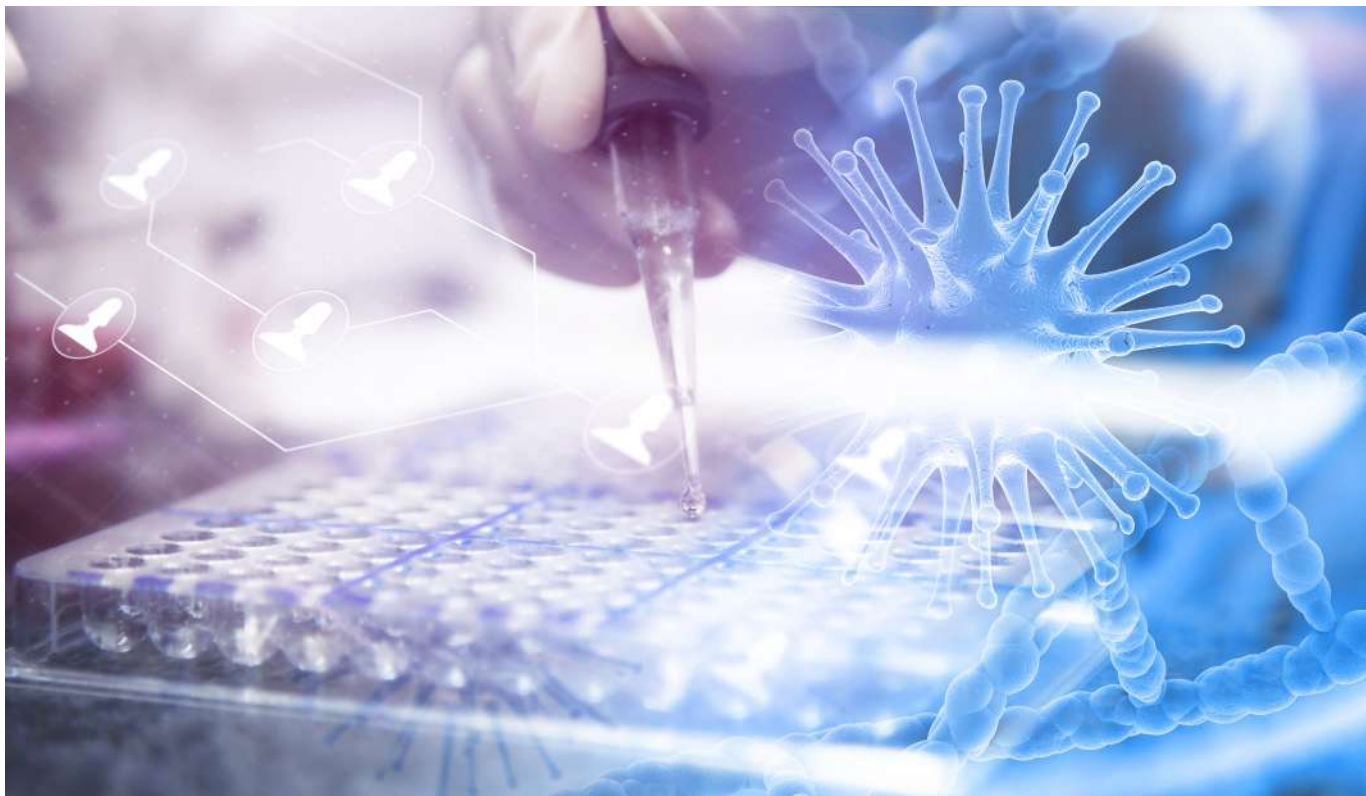
The solution: the user needs to perform the verification correctly onsite. What may feel like an unattainable goal due to the complexity of process validation, however, is simplified by the simple fact that the test is already considered to be validated. All that is required of a user is to verify that the already validated endotoxin test is suitable for the onsite products. The purpose of this document is to provide the user with the correct regulatory citations and guidance needed to give confidence in understanding the requirements of validation for the Bacterial Endotoxin Test according to USP compendial methods and advice, and FDA guidance.

### Process Validation

Page 4 of the FDA's "Guidance for Industry: Process Validation: General Principles and Practices" states, "Process validation is defined as the collection and evaluation of data, from the process design stage through commercial production, which establishes scientific evidence that a process is capable of consistently delivering a quality product." When a pharmaceutical or medical device manufacturer thinks of "validation," this all-encompassing definition is what comes to mind. The somewhat vague definition is what allows the scope of process validation to apply to every area of the drug or device manufacturing process. However, the document does provide a clean division of process validation into two distinct categories. Page 10 states, "Process validation includes facility validation and process performance validation."

### Facility Validation

Facility validation will fall completely out of the scope of Bacterial Endotoxin Testing. The Bacterial Endotoxin Test only has a few direct, basic facility requirements such as climate control and adequate shelter from direct sunlight. However, a site that is manufacturing a product to endotoxin-free standards



will need facility validation. Page 10 of the FDA document states, "Proper design of a manufacturing facility is required under part 211, subpart C, of the cGMP regulations on Buildings and Facilities. It is essential that activities performed to assure proper facility design and commissioning precede PPQ." Although not dealt with directly in BET validation, it is crucial that this portion of process validation is completed before the Process Performance Validation (PPQ), which includes endotoxin testing, proceeds. However, a client that is looking to implement the BET as a new test or a replacement of a previous technique should not need to change anything regarding their existing Facility Validation.

#### **Process Performance Validation/Qualification (PPQ)**

The Bacterial Endotoxin Test is one of the pieces that make up the PPQ. Page 11 of the FDA guidance on process validation explains that PPQ is the general name given to the combined validation of "the actual facility, utilities, equipment (each now qualified), and the trained personnel with the commercial manufacturing process, control procedures, and components to produce commercial batches. A successful PPQ will confirm the process design and demonstrate that the commercial manufacturing process performs as expected." One can think of the PPQ as the aggregate of all method validations needed for the quality control checks in the manufacturing process. On page 13, the guidance explains that PPQ includes "the validation of analytical methods used in measuring the process, in-process materials, and the product." Of course, one of these analytical methods is the Bacterial Endotoxin Test.

#### **Analytical Method Validation (Non-compendial Analytical Procedures)**

The manufacturer must provide a method of validation for every test they are using. The requirements for this validation are now outlined in the FDA guidance on "Analytical Procedures and

Methods Validation for Drugs and Biologics." Page 7: "Analytical method validation is the process of demonstrating that an analytical procedure is suitable for its intended purpose. The methodology and objective of the analytical procedures should be clearly defined and understood before initiating validation studies." The document provides a detailed description of potential objectives and tests to perform to ensure an analytical method is performing to its intended purpose. USP 1225 parallels these requirements: "Validation of an analytical procedure is the process by which it is established, by laboratory studies, that the performance characteristics of the procedure meet the requirements for the intended analytical applications."

It is at this point that many users of the Bacterial Endotoxin Test misunderstand the requirements for adopting or converting to a new test reagent. Because they either outsource the entire test itself or portions of the validation for in-house testing, they believe that adopting a new in-house test reagent and equipment will require them to perform a completely new Analytical Method Validation. However, because the Bacterial Endotoxin Test is a compendial method outlined in USP 85, the FDA considers the Bacterial Endotoxin Test to be an already validated test method. As a result, users do not need to perform an Analytical Method Validation but can proceed with a Compendial Analytical Verification.

#### **Compendial Analytical Verification/Verification of Compendial Procedures**

When either adopting a new or pre-existing reagent in a compendial procedure, this step of Compendial Analytical Verification is what is required of the user. The FDA document states, "The suitability of an analytical procedure (e.g., USP/NF, the Official Methods of Analysis of AOAC International, or other recognized standard references) should be verified





under actual conditions of use. Information to demonstrate that USP/NF analytical procedures are suitable for the drug product or substance should be included in the submission and generated under a verification protocol.” There is no need to validate the method itself, to determine its adequacy for the test, or even to perform a comparison study with an already-existing method (although that is often valuable for internal evaluation).

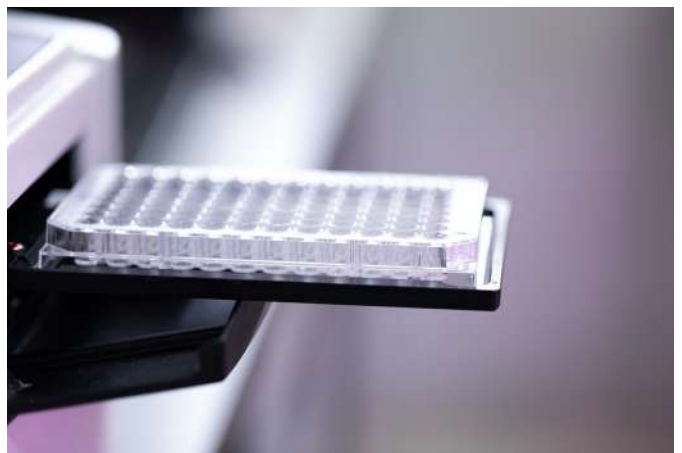
*The requirements are simply to verify the suitability of the method for the specific drug under actual conditions of use.*

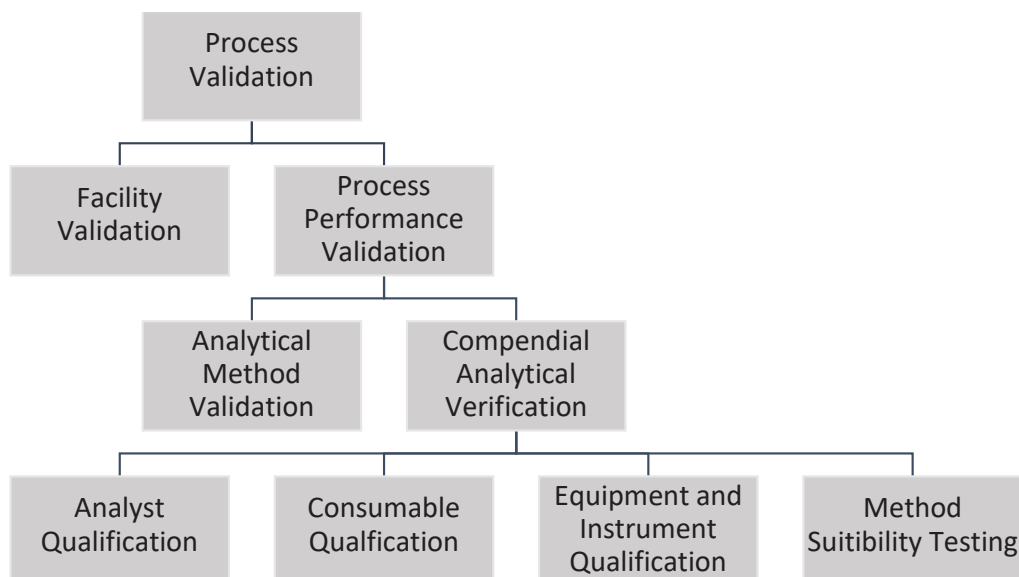
The USP chapter 1225 details what this compendial verification may look like, “Verification requirements should be based on an assessment of the complexity of both the procedure and the material to which the procedure is applied... Only those characteristics that are considered to be appropriate for the verification of the particular method need to be evaluated.” Although this may seem ambiguous, leaving the question open of how much of the Analytical Methods Validation steps need to be adopted for the BET test verification, the USP clearly outlines what is needed. USP chapter 1085 specifies that for

the Bacterial Endotoxin Test, this verification of compendial procedures includes analyst qualification, consumable qualification, equipment and instrument qualification, and method suitability qualification.

### **Analyst Qualification, Consumable Qualification, Equipment, and Instrument Qualification**

These three portions of the BET verification are the preparatory tests needed to be in place before the method suitability qualification. The following from USP 1085 regarding analyst qualification, “training for performing any BET involves demonstration of acceptable proficiency for both sample preparation and assay method(s).” For consumable qualification, “use apparatus that is shown to be free of detectable endotoxin and does not interfere in the test.” For equipment and instrument qualification, “all instrumentation and equipment used in the performance of an LAL test... should be qualified using proper scientific standards and according to approved protocols. Incubating plate or tube readers should reference a user requirement specification (URS), an installation qualification (IQ), an operational qualification (OQ), and a performance qualification (PQ).”





When providing proof of these qualifications, calibration certificates, CoA's, and IQ/OQ/PQ documents are attached to the method of suitability testing for the product.

### Method Suitability Testing

Finally, the actual verification required for a new user of the Bacterial Endotoxin Test is Method Suitability Testing. This is found in both USP 85 and expanded on in USP 1085 and includes the calculation of the endotoxin limit and MVD, the assurance of the standard curve test, and the interfering factors test. It is this process that is outlined in the following attachments. The new client has the assurance that the Bacteria Endotoxin Test is already considered to be validated when using an FDA-licensed reagent such as PYROSTAR™ ES-F. They also have the assurance that these considerations found in USP 85 are all that need to be addressed when either adopting a new test method or switching test methods.

If a user is concerned that switching between gel clot reagent to a quantitative reagent or switching manufacturers in reagent or instrument will require them to perform a PPQ since one of their analytical methods has changed, then they can be assured by the words in the opening paragraph of USP 85 that from a regulatory standpoint, nothing has changed in terms of their Analytical Methods Validation: "There are three techniques for this test... Proceed by any of the three techniques for the test."



### Conclusion and Introduction to the Following Documents

If you are considering changing your LAL reagent or technique due to failed results with a previously outsourced validation or to adopt a better BET technique, you have the assurance that the FDA and the USP consider this a simple change in the reagent. If you are considering adopting the LAL reagent for BET testing, you have the assurance that the FDA and USP already consider the method you are adopting as compendial. Once this is understood, your confidence can arise that you are following a fully compliant and compendial method. To aid in your risk assessment and preparation for performing the appropriate suitability assessment based on the USP and FDA requirements, FUJIFILM Wako provides a full range of endotoxin-specific LAL reagents. FUJIFILM Wako can support your validation by providing example procedures and reports that can then be used as a part of the submission of a successfully validated Bacterial Endotoxin Test.

### REFERENCES

1. FDA. Guidance for Industry: Process Validation: General Principles and Practices
2. FDA. Analytical Procedures and Methods Validation for Drugs and Biologics
3. USP <1225> Validation of Compendial Procedures.
4. USP <1226> Verification of Compendial Procedures.
5. USP <1085> Guidelines on the Endotoxins Test
6. USP <85> Bacterial Endotoxins Test



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